Establishment of engineered cell-based assays mediating LAG3 and PD1 immune suppression enables potency measurement of blocking antibodies and assessment of signal transduction

Bhagyashree Bhagwat, Holly Cherwinski, Manjiri Sathe, Wolfgang Seghezzi, Terrill K. McClanahan, Rene de Waal Malefyt, Aarron Willingham

PII: S0022-1759(17)30495-7
DOI: https://doi.org/10.1016/j.jim.2018.02.003
Reference: JIM 12411

To appear in: *Journal of Immunological Methods*

Received date: 21 November 2017
Revised date: 27 January 2018
Accepted date: 6 February 2018

Please cite this article as: Bhagyashree Bhagwat, Holly Cherwinski, Manjiri Sathe, Wolfgang Seghezzi, Terrill K. McClanahan, Rene de Waal Malefyt, Aarron Willingham , Establishment of engineered cell-based assays mediating LAG3 and PD1 immune suppression enables potency measurement of blocking antibodies and assessment of signal transduction. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Jim(2017), https://doi.org/10.1016/j.jim.2018.02.003

Establishment of engineered cell-based assays mediating LAG3 and PD1 immune suppression enables potency measurement of blocking antibodies and assessment of signal transduction

Bhagyashree Bhagwat[1], Holly Cherwinski[1], Manjiri Sathe[1], Wolfgang Seghezzi[1], Terrill K. McClanahan[1], Rene de Waal Malefyt[1], Aarron Willingham[1]*.

[1] MRL, Merck & Co., Inc., Palo Alto, CA, USA

**corresponding author*
901 California Ave, Palo Alto, CA 94304
650-496-1284
aarron.willingham@merck.com

ABSTRACT

LAG3 is an important regulator of T cell homeostasis and studies in mouse tumor models have demonstrated that simultaneously antagonizing LAG3 and PD1 can augment tumor-specific T cell responses and induce tumor rejection. The combined use of LAG3 antagonist antibodies with established anti-PD1 therapies is currently being evaluated in human clinical trials. A functional assay for human LAG3 was developed by co-culture of a Jurkat T-cell lymphoma line overexpressing LAG3 with a Raji B-cell lymphoma line in the presence of staphylococcal enterotoxins. Reversal of LAG3 repression was measured as an increase in IL-2 production or NFAT activation in response to treatment with MK-4280, an anti-human LAG3 antagonist antibody. Changes in cytokines, chemokines, and other mRNA transcripts were in agreement with published in vitro and in vivo models for LAG3 biology which highlights the physiological relevance of the Jurkat functional assay. Additional engineering of PD1 and PDL1 components into the LAG3 assay resulted in a bi-functional assay that is capable of inducing a 10-fold response to individual antibodies blocking either PD1 or LAG3. Importantly, when MK-4280 and pembrolizumab were combined to block both pathways, a synergistic 50-fold increase in response was observed.

KEYWORDS

LAG3; PD1; MK-4280; pembrolizumab; Jurkat; cell-based